

Microbial Finishing at DOE JGI/PGF: Sequencing Difficult DNA Templates

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The US DOE Joint Genome Institute's (JGI) mission is to provide the scientific community with high-quality finished genomes. Approximately 300 microbial genomes are currently in the JGI pipeline and to date, 79 have been completed. The objective of the Microbial Finishing laboratory is to process sequencing reactions in order to increase the quality of reads, to close physical gaps and/or sequence gaps. Most genomes contain complex regions which are difficult to sequence with standard protocols, so the laboratory must use a multitude of techniques specialized for each project. Problematic regions include, for example, GC-rich areas, hairpin loops, homopolymer stretches, and tandem repeats of variable length. Gap closure in such regions is expensive as well as time-consuming, since it requires extensive troubleshooting strategies. Approaches include, optimizing reaction conditions, applying various sequencing chemistries, and additional manual editing. In an effort to greatly reduce the amount of areas requiring special analysis, genomes with >65% GC content are processed with a four step-approach: 5% DMSO, Sequence Finishing Kit (SFK), PCR, and shatter libraries. As a result of this strategy, JGI/PGF's Microbial Genome Finishing Group has been able to complete a number of complex microbial projects, such as, *Frankia* (~75% GC-rich) and *Thermobifida fusca* (~68% GC rich).

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